THE VETERINARY JOURNAL

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Vol. 104 November, 1948 No. 11

EDITORIAL

THE USE OF NEW PRODUCTS

THE Veterinary Profession is sometimes the subject of criticism for not being in a better position to control the major diseases affecting livestock. It has to be admitted that our knowledge of all the circumstances which lead to the development and spread of disease is still incomplete. At the same time, however, it can be claimed that the modern methods of control are surely and gradually making a marked impression upon the incidence of some of the diseases of economic importance throughout the country. While we may boast of some of the achievements of recent years, we must also bear in mind the excellent work carried out by our predecessors in actually ridding the country of major scourges like cattle plague and glanders; and their efforts were made and were fruitful in spite of the lack of present-day knowledge. Observations made many years ago and, from them, the application of control measures are now shown to be sound in the light of more modern scientific knowledge. There is cause to marvel at the excellent results achieved without all the appliances and information which we now possess and which we now consider essential.

This does not mean, of course, that we have not progressed in more modern times; far from it, for our progress has been more rapid and will continue to develop quickly as more and more scientific work is carried out. Research work is the keynote to progress, for the application of the results of such work to the problems in the field follows as a matter of course. New fields are being continually explored and the findings of science are used wherever they can be expected to be of value. It is not uncommon to find that the results of research work in branches of science, quite different from biology, have a definite place in the control of animal health and disease. Our research workers must be on the look out all the time for the results of the efforts of others carrying out investigations and experiments on subjects which, at first sight, might appear to be quite foreign to our own subject. The encouragement now given to research work in the various and numerous directions is producing results of far-reaching importance. We welcome this encouragement and hope that it will influence men

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and women in considerable numbers to take up research work as a career and so to add more rapidly the knowledge so necessary for our better understanding of the problems of animal health and disease which await solution.

Nothing is more outstanding in our work than the use made of chemical agents in the prevention and treatment of diseases. We are taking full advantage of the various compounds as they are introduced and we must continue to do so. It does not follow, of course, that all or even many will find a place in our work, but they must be given a trial. The successful use of penicillin in the treatment of some forms of bovine mastitis, for example, has altered our method of control of one of the important economic diseases of dairy cattle. The application of drugs of the sulphonamide series has been of marked value in the control of coccidiosis in chickens. Without the compound, phenothiazine, the control of infestation with some species of round worms in the alimentary canal of sheep would not be so easily accomplished. The marked effects of D.D.T. and benzine hexachloride have altered our methods of control of the external parasites of sheep, some of which are carriers of infections of importance. These are but a few examples of the results of modern research work in which advantage is taken of the findings of workers in other branches of science. As time goes on and new products are elaborated, we will continue to apply them to our work in the hope that some of them, at least, will find a place in the control of diseases of livestock and other animals.

GENERAL ARTICLES

RECENT DEVELOPMENTS IN IMMUNITY, MORE PARTICULARLY FROM THE STANDPOINT OF VETERINARY MEDICINE, AND WITH ESPECIAL REFERENCE TO EGYPTIAN CONDITIONS*

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(*Continued*)

THE swine fever virus present in the shed blood of an infected pig is subjected to a great variety of influences, of which the following are possibly some of the most important:

(a) If the blood has become accidentally contaminated with bacteria, putrefaction will soon set in, especially if the blood is kept at body temperature. Putrefaction is known to have a rapidly destructive effect upon the virus. The presence of crystal violet in the blood would inhibit usually the onset of the putrefaction. To supplement the action of the crystal violet, a small concentration of ethylene glycol is now recommended for addition to the defibrinated blood before incubation at 37° C.

(b) Allowing that the blood remains free of bacterial contamination, changes would still take place very quickly in it which are of nearly the same order as putrefaction. These are in large part conditioned by the presence of a great variety of enzymes in the blood which bring about autolysis of the red blood corpuscles and death and disintegration of the leucocytes and a marked change in the protein constituents of the plasma, all tending somewhat to change the medium in reaction towards the acid side. The presence of crystal violet in the medium would inhibit the action of many of these enzymatic mechanisms, prevent the onset of a strongly reducing condition within the medium, and generally prevent the virus from undergoing lysis from the action upon it of the enzymes liberated in its environment.

(c) The popular conception of "Heat" is strongly linked to the sensory perceptions of the human subject as a homiothermic animal, the temperature of whose internal tissues, as in the case of other homiothermic animals, being regulated in the close vicinity of 37° C. during life. Unless those tissues are maintained at that temperature, life of the individual itself soon ceases.

Now, that conception may easily lead to a false appreciation of what are the heat requirements of living agents other than the intact homiothermic animal for survival.

In the case of the filterable viruses generally, for example, it has been found that their survival is prolonged in measure as the temperature at which they are maintained is lowered. It has been proved in the case of many of them that they can be suspended in a medium which is lowered in temperature to that of liquid air and then raised again to thawing point, and this process of alternate low

* An Essay originally prepared at the request of the Examiners for the Degree of Ph.D. in the Faculty of Science, Fouad I University, Cairo.

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temperature freezing and thawing repeated several times, without diminishing the content of the medium in live virus. To preserve the more fragile viruses, the standard procedure now adopted is that of drying in vacuo at very low temperatures (" freeze-drying ") and storage afterwards in a state of complete desiccation in vacuo, so that the virus is not exposed to the destructive action of humidity and atmospheric oxygen.

A temperature of 37° C. may therefore represent for the swine fever virus, as well as for several other viruses, an " inactivating temperature," provided (a) it does not have access to a sufficiency of suitable host cells in a state of normal metabolism, in which event the conditions are ideal from its proliferation; or (b) the material in which it is suspended outside the living host is not progressively undergoing a series of changes—putrefactive, enzymatic, etc.—which will lead to rapid lysis of the virus particle and, hence, to destruction of its immunogenic properties.

In the case of some other viruses, such as that of foot-and-mouth disease, where inactivation of virulent blood at 37° C. in the presence of crystal violet has been studied, the mechanism seems to have been proved to be as outlined above—namely, that actually it is one of " Heat Inactivation," the crystal violet functioning as an inhibitory agent against lytic action upon the virus.

Where the immunogen is available in a state of purity, the above complications arising from influences within the ambient do not interfere with our study of the inactivating process.

Hence, in the case of " formalin " (40 per cent. formaldehyde, HCHO) inactivation the process appears to be a simple aldehydation of some of the amino acids within, or on the surface of, the protein molecule of the immunogen. Often, this process, even when carried out to an intense degree, does not diminish the immunogenic property of that molecule. For example, in the case of the virus of cattle plague it has been found advantageous to employ a concentration of formalin amounting to about 1 per cent. in inactivating virulent spleen or lymphatic gland tissue, whereas in the case of foot-and-mouth disease a concentration exceeding 0.1 per cent. acting upon virulent lymph has been found to be decidedly deleterious.

Much the same applies in the case of the bacterial immunogens. In the inactivation of *Brucella abortus*, for example, prior to using it as a useful immunogen in the form of the inactivated bacilli suspended (" enrobed," to use the French term) in a non-absorbable menstrum (such as lanoline and beeswax), a concentration of 1 per cent. formalin can be safely used. As already mentioned, in the inactivation of the cholera vibrio a much lower concentration is employed. Some of the objections towards using a high concentration of formalin reside in the irritant effect of an excess of this chemical upon the host's tissues after inoculation. The excess, as was proved several years ago in the inactivation of the dog distemper virus, can be readily neutralised with an equivalent quantity of ammonia, to form urotropine, which is non-irritant.

The degree of heat required for inactivation, where heat is employed, varies greatly. Usually, it has been considered that the degree of heat should be such as is sufficient, and only just sufficient, to kill the immunogen—in the case of the

vegetative forms of bacteria generally, about 56° C. for 1 hour or less, or 60° C. for 20 minutes or less, or in the case of bacterial spores in pure suspension (as in the case of *Clostridium chauvoei,* cited above) at 100° C. for about 1 hour. There is no strict rule in this matter, however, for as already stated, several pathogens lose almost entirely their immunogenic properties immediately they are killed by heat, while others preserve them remarkably well.

It would be beyond the scope of an essay to enter further into a discussion of the action of the great variety of chemical and physical agents that have been employed for inactivation. A very large field of work here remains open for further exploitation. Generally, as appears from our own discussion, the principles involved are two-fold—namely: (a) to employ some agent which will ensure against disruption of the protein molecule through lysis; and (b) to select an agent that will not cause brutal or irreversible damage to the molecule through denaturation.

The Routes of Injection of the Immunogen

This is not invariably a matter of indifference. In the two examples which we selected for illustration at the outset, the route of injection prescribed in both cases was the subcutaneous one.

Circumstances, however, may decide that some other route is preferable. In the case of active immunisation with diphtheria toxoid, the route selected was the intracutaneous ("intradermal") one.

For some purposes, as when an unsightly or painful local inflammation arises after subcutaneous inoculation, leading to necrosis of the overlying skin, followed by suppuration, the intramuscular route may be preferred.

The intraperitoneal and intravenous routes have been tried in some cases, but usually they secure no advantage. In fact, in a few cases the intravenous route has been shown to be the least efficacious inasmuch as it leads to the most rapid elimination of the immunogen from the body.

Almost invariably, no appreciable degree of immunity can be evoked by administering an inactivated immunogen by ingestion.

This does not mean to say that the cells lining the alimentary tract are completely indifferent to the action of immunogens, in the same way as the surface layers of the cutaneous integument are indifferent.

That the lining cells of the alimentary tract are themselves highly sensitive, sometimes at least, to the action of immunogens is illustrated by the wide variety of diseases classified as diseases of childhood or diseases of the new-born in which the individual becomes affected with micro-organisms which are exalted to the status of pathogens when they come in contact with the alimentary mucosa of the very young, but which are depressed to the status of sub-pathogens or commensals in the adolescent and adult.

Recent research has shown that in large part this phenomenon is related to the immunity which the mucosal cells acquire soon after birth as the result of the ingestion of immune antibodies in the milk of the suckling mother, the colostrum being particularly rich in this respect. These immune antibodies may not have

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been able to traverse the cotyledonary placenta during fœtal life, but are able to traverse the alimentary mucosa during extra-uterine life.

As has already been stated, advantage has been taken of this knowledge in the protection of lambs against lamb dysentery, where it would be very difficult to intervene early enough after the birth of the lamb to establish an immunity by inoculation subcutaneously with an anti-serum. Instead, the mother is immunised during pregnancy with an inactivated immunogen and, subsequent to purturition, the lamb ingests a sufficiency of immune antibody in the mother's colostrum to protect it from natural attack.

Much the same kind of phenomenon arises when calves suck the milk of a mother immune or partially immune towards cattle plague. Calves usually present here a higher degree of resistance than adults and this resistance seems to be related to a passive immunity acquired during the sucking period. Moreover, the processes in the case of this disease are not yet quite clear.

Before an inactivated immunogen can come into intimate contact with the cells of the alimentary mucosa, it has to be exposed to the action of the digestive juices as well as to bacterial decomposition in the unassimilated ingesta.

The superiority of the intradermal route over the subcutaneous route immunisation with diphtheria toxoid seems to be related to two factors. Firstly, within the dermal tissue the adsorbed toxoid is less liable to become absorbed into the general circulation and quickly excreted before an active immunity is evoked. Secondly, the lymphatics of the dermis are in close connection with the adjacent lymph nodes ("lymphatic glands"), so that most of the liberated immunogen very soon finds its way almost entirely into immediate contact with the lymphocytes in these nodes, which are *par excellence* the cells capable of responding by the production of immune bodies. Cells elsewhere in the body which are histogenetically evolved from the lymphocytes and have the same property of responding by the production of immune antibodies were grouped together by Aschoff under the name Reticulo-Endothelial System, and this conception has now found widespread acceptance among pathologists and immunologists. The densest aggregation of a system of this kind in the body is to be found, besides in the lymph nodes, in the spleen, but wherever in the body cells of similar origin are to be found—such as, for example, the Kuppfer cells of the liver—tissue possessing the property of immunogenic response is available. During the recent preliminary experiments on cattle plague immunisation in Egypt (Edwards, 1946), it was clearly shown that a strain of goat virus only moderately attenuated (Kabete strain) was capable of producing a high mortality rate after intradermal inoculation (by scarification inside the ear), even though, on comparable cattle, its effects after subcutaneous inoculation were non-lethal. This experience is adverted to parenthetically for the reason that it illustrates a difference between the intradermal and subcutaneous routes, in the former route enhancing the virulence of an infective agent. Henderson recently has shown that the dermal tissues are in very close and direct relation with the adjacent lymph nodes through the connecting lymphatic vessels, so that after intradermal inoculation of colouring materials like trypanblau or Indian ink, or of the virus of foot-and-mouth disease, the greater part of the

agents finds its way within a few seconds into the lymph nodes. Likewise, the introduction of an inactivated immunogen, like APT of diphtheria intradermally, would quickly provoke a massive immunity response from the mass of "reticulo-endothelial" tissue represented by the lymphocytic tissue of the lymph nodes.

When an immunogen, on the other hand, is injected subcutaneously or intramuscularly, the great mass of tissue with which it comes in contact, such as subcutaneous fascia or muscle fibres, are poor in immunogenic response.

The Successive Stages in the Evocation of Immunity after Inoculation with an Inactivated Immunogen: A—The Negative Phase

Present-day knowledge upon this phenomenon can, perhaps, be best illustrated if we compare the reactions which take place in the course of the Tuberculin Test as now commonly performed for the diagnosis of tuberculosis, and the Schick Test as performed to ascertain the sensitivity of the human subject towards diphtheria.

Both tests consist in the inoculation of the products of the growth of the specific micro-organisms into the skin.

In the case of the tuberculin test, the injection of a minute dose of extracted tuberculo-protein provokes, in an infected subject, the appearance of an œdematous painful swelling, which is usually accompanied with a febrile response, within 48-72 hours after injection. In a non-infected subject the response, local and general, is negligible.

In the case of the Schick Test, the injection of a minute dose of diphtheria toxin into the skin in a carrier or immune subject is followed by hardly any noticeable local change, while in a susceptible subject there arise well marked signs of local inflammation.

At first sight, the reactions considered positive in these two cases would appear to be the opposite of each other.

On closer examination, we find the following sequence of changes:

(a) When the tuberculo-protein is injected into the dermis of an infected subject, the cells lining the lymphatics respond actively and a first stage in the response is the liberation from the cells of histamine or histamine-like substances.

(b) These liberated substances have a profoundly toxic effect upon the tissues, leading to failure of the closed lymphatic system to permit of the restrained normal diffusion of lymph through the walls of the vessels, with consequent extravasation of lymph into the intercellular spaces.

(c) If the tuberculo-protein has been injected, on the other hand, into the dermis of a healthy but susceptible subject, the initial response mentioned above does not take place and no entravasation of lymph occurs.

(d) When diphtheria toxin is injected into the dermis of an immune subject, the initial response mentioned above also takes place, but immediately the toxic substances liberated are neutralised by immune antibody circulating in the lymph of the subject.

(e) When the toxin, on the other hand, is injected into the dermis of a susceptible subject, the initial response, in large measure, occurs, but absence of

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a sufficient concentration of circulating antibody prevents neutralisation, so that the capillary walls, and here particularly of the capillary blood vessels, are severely damaged.

In the case of tuberculous subject, therefore, a partial immunity has developed, but it has not become strong enough to neutralise the toxic effects of the products of reaction between the host cells and the tubercle bacillus. The cells, on the contrary, which are sensitive to these products, are rendered hypersensitive, or allergic.

In the case of a subject that is resistant to invasion with the diphtheria bacillus or the effects of its toxin, the allergy has passed onwards to be transformed into an immunity, when an adequate concentration of neutralising antibody is discoverable in the body fluids.

It would seem that in the negative phase occurring after inoculation with an inactivated immunogen, the sensitive tissues in the host are undergoing a preliminary, analogous, stage of allergy.

Where, as in several known disease states, the sensitive tissues have not been able to pass beyond this stage, they remain in a continuous state of allergy.

A recent discovery has been that this state of allergy can be transferred passively to another subject. If blood serum is taken from a subject undergoing the allergic response and injected into another subject, the latter is made temporarily hypersensitive to the invasive effects of the antigen.

The above illustrations may tend, however, to over-simplify exposition of the complex processes occurring throughout the body, leading to the establishment of an immunity.

Beside immune antibody, several more properties that, depending upon their manifestations have gone under different names—agglutinins, complement-fixing substances, precipitins, opsonins, aggressins—can be demonstrated to be arising in the body fluids of the host.

What the relation is between these properties and actual immunity is, at the moment, most obscure. Certainly they are not clearly demonstrable until a considerable interval, amounting to several days, has elapsed after inoculation with the antigen. A large amount could be written around each of these properties, but in this essay they need to be reviewed only in so far as they afford an indication of the protection acquired by the host against an infection, i.e. an immunity.

In a horse suffering from glanders, for example, a very high agglutinin content may appear in the blood serum of an infected subject, but, nevertheless, the clinical course of the disease may pass inevitably to a fatal termination. Similarly, in the case of human typhoid and the other salmonelloses, the brucelloses and several other infections, a strong agglutinin response does not indicate effective resistance against them. In the case of some infections, such as the pasteurelloses, effective resistance can be established without the production of a strikingly increased concentration of agglutinins.

That agglutination of bacterial invaders must be a step in the direction of affording the tissue cells better opportunities to compete with them is fairly

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obvious. The role of precipitins is not quite so obvious. The role of the opsonins in promoting phagocytosis seems to be clearly established. The role of the aggressins is almost wholly obscure. (See, however, later section on the role of certain enzymes acting as "spreading factors" in promoting the virulence of organisms like *Clostridium welchii* and the cholera vibrio.)

The Successive Stages in the Evocation of Immunity after Inoculation with an Inactivated Immunogen: B—The Positive Phase

In the light of what has been said above, the positive phase can be regarded as the stage when, after partial exhaustion of the normal immune antibody content of the host during the negative phase, the sensitive responding cells of the reticulo-endothelial system are stimulated to the production of immune body far in excess of what is required for the disposal harmlessly of the inoculated inactivated immunogen.

What the actual physico-chemical processes are that bring about this response are still under investigation, though a few of them have already been touched upon. In some measure, they are related to genetic, nutritional and environmental peculiarities. For example, in a given species, it has long been well known that some individuals acquire a high degree of immunity following inoculation with a specific immunogen, while others are left almost unprotected. Usually it is difficult to secure a good immunity response in animals in a low nutritional state. That the animals within one area appear to respond better than those in another area seems to be related more to genetic differences than climatic influences.

It has already been stressed that no uniformity in the intensity of the positive phase is to be expected after inoculation with the different inactivated immunogens derived from different bacterial groups or in the different species of animals following inoculation with a given immunogen.

The Waning of the Active Immunity following upon Inoculation with an Inactivated Immunogen

Whatever the degree of immunity arising during the positive phase of the response, it afterwards gradually wanes. The rapidity with which the immunity wanes varies enormously, depending upon a number of circumstances many of which are now well known, although their mode of operation is still but imperfectly understood.

Perhaps these various circumstances can again be best illustrated if we select the example of cattle plague and the variety of phenomena which may arise after an attempt has been made to vaccinate a susceptible ox against this disease by means of an inactivated vaccine.

The source of the inactivated vaccine is the spleen and the lymphatic tissue of a highly susceptible ox killed at the height of the reaction—namely, about the fifth or sixth day after inoculation with the cattle plague virus. The tissue pulp is inactivated either after suspension in 33-50 per cent. glycerine and maintenance for a few days at 37° C. or else by treatment of watery suspension (1 : 3 in water)

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with 0.7-1.0 per cent. formalin and keeping at room temperature for about two days, followed by storage in the cold.

Subcutaneous inoculation of an adult ox with 1-2 grams of the inactivated tissue is usually followed in about 12 days with the onset of a powerful immunity. If the tissue material has contained a low concentration of virus before inactivation, the resulting immunity will be correspondingly weak. A weak vaccine will be obtained if cattle of relatively low susceptibility are selected as donors and if the tissue pulp is harvested at a time when the concentration of virus in it has fallen very low. Usually, adult cattle of high susceptibility are more powerfully immunised with the inactivated vaccine than are cattle of low susceptibility.

Now, if cattle that have been vaccinated with a good brew of the inactivated vaccine are tested for their immunity by inoculation of fully virulent blood at varying intervals it will be found that the degree of protection gradually diminishes.

In the case of vaccinated adult cattle, the protection will be found insufficient usually to ward off an attack of fatal cattle plague if the interval is extended beyond eight months. In the case of vaccinated calves, the interval is much shorter and usually does not extend beyond one or two months. The interval is longer if the mode of testing is exposure to natural infection instead of artificial inoculation, and in the case of adult cattle it has sometimes been found to extend to about two years.

The decline in the immunity, in any case, is not an abrupt process but takes place gradually.

Assuming that a test of the immunity is carried out by inoculation of the "challenging" dose of virulent blood at a short interval—say, one to two months—after vaccination, any one of the following four syndromes may be observed to occur:

(a) The animal may show no reaction whatever, thermal or clinical. Tested much later—say, in two years—with a challenging dose of live virus, the animal reacts just as if it had not been inoculated at all with the first challenging dose of live virus. In other words, at the time of inoculation with the first challenging dose the immunity conferred by vaccination was still so powerful that the virus failed to obtain a foothold, i.e. to "establish" itself, in the tissues of the vaccinated animal.

(b) The animal may show no distinct reaction, thermal or clinical. Tested much later (as above), it will, however, be found to be fully immune. If some blood had been withdrawn from the animal from time to time during the days following inoculation with the first challenging dose and inoculated into susceptible experimental cattle, proof would be obtained that virus was actually circulating in the blood of the animal after inoculation of the challenging dose. In other words, the virus had then succeeded in establishing itself in the tissues, and the result was that the animal had acquired a solid and durable immunity. The reaction following the first challenging dose was, therefore, a "symptomless," afebrile, or "blocked-out" one.

(c) The animal may show a distinct thermal reaction but no distinct clinical symptoms. Tested much later (as above), it will be found, as expected, to be

fully immune. The virus in this case had obviously established itself after the first challenging dose. The reaction could be classed as a " febrile but clinically symptomless " and therefore " occult " one. In the economic field, where a " vaccine-virus " method of immunisation was adopted, a reaction of this kind would be the ideal one, because it would furnish the clinical observer readily with evidence that the virus had become satisfactorily established while, at the same time, it entailed no risk to the life of the animal.

(d) The animal may show a distinct thermal reaction as well as a clinical reaction, which may vary greatly in intensity, from an exhibition of very mild clinical symptoms such as temporary inappetence, some lachrymation and perhaps, on close examination, mild buccal lesions, with some looseness of the bowels, to severe disturbance, well marked diarrhœa, resembling more or less distinctly the natural disease. Even an observer well acquainted with the symptoms of cattle plague would not suspect the very mild symptoms to belong to cattle plague if he were not forewarned. Cases of this kind, therefore, are classed still as " occult " forms of the disease, with " inapparent " cattle plague symptoms. Obviously following the onset of such symptoms the virus has become well established in the tissues and the resulting immunity would be lifelong.

A very grave danger is, however, represented by all such cases in which the symptoms remain " occult " or " inapparent " inasmuch as the affected cattle are capable of transmitting virulent, and fatal, infection by contact to healthy, non-immune cattle kept in their vicinity.

That this danger is a very real one has now been proved abundantly both by laboratory experiments and from field experience. Where cattle are inoculated with an inactivated vaccine and cattle plague still lurks in their vicinity, the disease smoulders on, for an indefinite time, in an " occult " or " inapparent " form, among the vaccinated cattle, and a severe outbreak of cattle plague may occur at any time when the infection spreads from these cattle to healthy non-immune ones kept near them.

Hence, for epidemiological reasons, as well as on economic grounds, vaccination of cattle in endemic cattle plague areas by means of an inactivated vaccine has now been generally abandoned in favour of immunisation with an attenuated live virus (" goat-virus method ").

The experience in vaccination against dog distemper runs somewhat parallel. Here vaccination with inactivated vaccine evoked, it was found, an immunity of only short duration but it was, nevertheless, useful in conferring upon susceptible dogs a basal immunity sufficiently strong so that they could be inoculated 10-14 days later with the fully active live distemper virus without incurring grave risk of a very severe reaction (Laidlaw-Dunkin method). Here it was found most important to prepare the inactivated vaccine from the tissues of infected dogs and not from the tissues of ferrets, which are now largely used in distemper experiments. Passage of the virus into another host would seem to deprive it of immunogenic power, after inactivation, for the natural host. Much the same kind of phenomenon occurs in cattle plague, where passage of the virus into goats

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seems to deprive the tissues of the latter from providing a good inactivated vaccine for use on cattle.

The difficulty in regard to "balancing," as between inactivated vaccine and live virus, however, arises in the case of immunisation against dog distemper in much the same way as in the case of cattle plague. This difficulty, in the case of dog distemper, has now led to preference being given to the serum-virus method over the vaccine-virus method in general practice.

From what has been said above concerning the serious limitations in the use of inactivated vaccines alone in immunisation against cattle plague and dog distemper it might be concluded, all too easily, that in general "field" practice these vaccines are of very little value. It is true that in the case of a very large number of diseases, as has already been said, they are almost entirely valueless. Our present knowledge goes to show that, contrary to the belief which was gaining currency in the earlier part of the bacteriological era, with many diseases it is still impossible to devise a satisfactory vaccine. Even where, as in the case of pneumococcus pneumonia in the human subject, considerable strides had been made in our understanding of proper methods of vaccination, the advent of the sulphonamide drugs, and later of penicillin, into current therapeutic treatment has suddenly raised greater hope in this infection, at any rate, from chemotherapy than immunology.

However, in some classes of infection, remarkable results have been obtained from the use of inactivated vaccines, as our remarks upon immunisation against human diphtheria, lamb dysentery, and (within certain limits) swine fever already testify. Here, in these examples, the pathogen to be immunised against comprised a bacterial toxin, an anærobic bacillus and its toxins, and a filtrable virus, respectively; that is to say, pathogens of very widely varying characteristics. But while, for example, a useful immunity can be evoked against the filtrable virus of swine fever by means of an inactivated vaccine, nothing can be achieved throughout the whole range of diseases caused by variola-vaccinia group of viruses by means of inactivated vaccines. On the other hand, remarkable success has attended the use of inactivated vaccines in the case of human yellow fever and equine encephalomyelitis (in U.S.A.) when suitable strains of the viruses are grown in the developing chick embryo and inactivated by formalisation.

In a few cases the results following the use of inactivated vaccines turn out to be better in actual field practice than would be forecasted from the results of laboratory experiment.

For example, in the case of bovine hæmorrhagic septicæmia, caused by *Pasteurella boviseptica*, the customary method of immunisation, both in India and in Egypt, is by means of a heat-inactivated bacterial culture, and, on the whole, the method has given remarkably satisfactory results in field practice. Tested, however, in the laboratory, whether on susceptible cattle or on rabbits, by inoculation subcutaneously of a challenging dose of highly virulent live culture, the immunity evoked by the killed culture seems to be an extremely weak one. This has led some American workers (Kelser *et al.*) to have recourse to the

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inoculation of a better product, in their estimation, in the shape of an " aggressin," represented by the filtered œdematous fluid produced locally in cattle after subcutaneous inoculation with a suitably virulent strain of *Pasteurella boviseptica*. An even stronger immunity can be produced by the subcutaneous inoculation of an attenuated live culture of the organism simultaneously, at another site, with a dose of anti-serum.

However, there is no doubt that in the field the original heat-inactivated vaccine answers requirements, and the reasons why this happens appears to reside in two respects, which cannot be imitated in the laboratory.

Firstly, it is well known, in India, at any rate, that the endemicity of bovine hæmorrhagic septicæmia is seasonal as well as regional. In the endemic areas, outbreaks occur among young cattle—both ordinary cattle and (particularly) buffaloes—soon after the break of the monsoon season. That is, the endemic season lasts about three months, from the end of June until the middle of September. Protection is therefore required to be effective only for a relatively short period.

Secondly, the natural mode of infection in the case of bovine hæmorrhagic septicæmia is still unknown. Artificially, in the laboratory, it is impossible to transmit it by ingestion, even of massive doses of highly virulent culture. Whether it is transmitted by means of an insect vector, as in the case of human plague (caused by a closely allied organism, *Pasteurella pestis*) has not yet been proved. Anyhow, in the natural disease, provided the analogy with human plague holds good, initial infection would be caused by an infinitely small dose of virulent organisms. In the case of human plague, the dose is represented by the regurgitated œsophageal culture in the infected rat flea, which gains entrance at the site of skin puncture. Now, in the laboratory, where the challenging dose is represented by the contents of a hypodermic syringe, the test employed to ascertain immunity is far more drastic and crude than in the case of natural infection.

Hence, protection is required of just sufficient degree to prevent the establishment in the tissues of a microscopic quantity only of virulent Pasteurella, and for a short period.

Both in the case of human plague and bovine hæmorrhagic septicæmia, as well as in fowl cholera (caused by *Pasteurella aviseptica*) the practice of evoking a low-grade immunity by inoculating of heat-killed cultures would therefore appear to be well founded.

It is clear already that in veterinary medicine a proper study of immunology is of much greater importance relatively than in human medicine. In both branches of medicine, the subject is of very great importance, but whereas in human medicine disorders affecting the individual subject are economically and æsthetically of very great importance, in veterinary medicine, on the other hand, it is those disorders which affect animals in the mass that are of the greatest importance, for purely economic reasons, while those affecting the individual subject only are of trivial importance in comparison.

Hence, a thorough understanding of preventive medicine—comprising immunology and epidemiology—is, in veterinary medicine, economically from the

general public standpoint more desirable than even good experience in clinical veterinary practice.

Immunisation by Means of Active or "Live" Immunogens

In an address given by the late Sir John Ledingham a few years ago at the Comparative Section of the Royal Society of Medicine, London, on Active Immunisation against the Virus Diseases, he declared, at the outset, that the Jennerian method of vaccination against human smallpox must still be regarded, after a lapse of over 150 years, as the most ideal form of vaccination hitherto achieved. It is now most difficult to imagine the extent and severity of the ravages caused among human populations before Jenner introduced his system of vaccination, which, in essentials, even with our enormous increase in knowledge upon the virus diseases and almost every other class of diseases and upon immunology, has undergone very little modification since his day.

Essentially the Jennerian method consists in the immunisation of the susceptible human subject by means of a virus which has become adapted naturally to the tissues of the ox, and in the course of that process has become greatly attenuated in virulence for the human subject. Hence, when it is inoculated by scarification into the skin of a human being it sets up no more than a relatively mild local reaction but the reacting subject is afterwards immune, usually throughout life, against attack by smallpox.

Rather surprisingly, until very recent years, very little serious attempt was made to ascertain whether a similar method of protection could be set up against other diseases.

It is true that Pasteur's own work revealed him to be fully cognisant with the essentials of the subject viewed from the standpoint of the Jennerian system, and the whole sequence of his work might be described as culminating in research into immunology more particularly from the standpoint of veterinary medicine, in the manner of the present essay. Thus, his first experience in immunisation was upon fowl cholera, where he found that fowls inoculated with an old broth culture of the causal microbe afterwards proved resistant to virulent infection. His next experience was upon swine erysipelas, where he believed he had found that passage of the causal microbe through a species other than the pig, namely, the rabbit, caused it to become attenuated in virulence for the pig, so that it could be safely used for the vaccination of pigs against the natural disease. Although, in this case, Pasteur had embarked upon a new principle in active immunisation—which had an analogy in the Jennerian system—the method was found to be rather too risky for use in ordinary field practice. Next came his remarkable researches in anthrax immunisation, which probably largely because an analogous method to what he discovered did not find immediate application in human medicine, has not been fully appreciated in its essentials even to the present day.

Pasteur's system of vaccination against anthrax comprised two inoculations. For both inoculations the vaccine used was a live culture of the anthrax bacillus, which had undergone progressive attenuation by cultivating it in broth at a temperature at which vegetative growth could still take place but sporulation was

prevented from occurring, namely, about 40.5 deg. C. After serial transfer (" passage ") in broth culture at this temperature the culture became depressed or " attenuated " in virulence until it reached a stage, firstly, when it did not kill all inoculated rabbits, but it killed all inoculated guinea-pigs, and, secondly, at a later stage in transfer, it did not kill all inoculated guinea-pigs but killed all inoculated mice. Rabbits, guinea-pigs, and mice therefore represented species of progressively greater susceptibility towards the anthrax bacillus.

As a first vaccine, the susceptible ox or sheep was inoculated with the culture that had undergone the greater degree of attenuation. As a second vaccine, the animal was inoculated with the vaccine that had undergone the lesser degree of attenuation. The second vaccine was inoculated at an interval of 10-14 days after the first vaccine. Pasteur's classical researches on anthrax immunisation were followed, as is well known, by his researches upon rabies immunisation (to which reference will be made later). Because of the importance of this last-named method of immunisation in the eyes of the medical profession and general public there has been a persistent tendency to regard it as Pasteur's crowning achievement, and the importance of his other pioneer work in immunology has been greatly overlooked. At this moment, it is of the highest interest to recall Pasteur's early researches, performed about seventy years ago, because an appreciation of them throws a flood of light on current problems of the greatest importance in veterinary preventive medicine.

Pasteur's researches on anthrax immunisation bring into relief two cardinal points in the practice of immunisation with a viable immunogen:

(a) He recognises gradations in susceptibility (and its converse, resistance) among host animals towards a given pathogen. In the particular case of anthrax, it happens that the three different species, rabbit, guinea-pig and mouse, represent progressive gradations in susceptibility, all of them being of greater susceptibility again than the common natural hosts, the ox and the sheep.

(b) He recognises the possibility of producing artificially, by appropriate means, gradations in the virulence of the given pathogen. In the case of anthrax, it happens that progressive gradation in virulence can be obtained by serial transfer in broth culture incubated at a certain temperature.

Recognition of these essentials enables us now to comprehend more clearly the rationale of some of the newer methods of immunisation that have obtained current use in human and veterinary medicine.

It is noteworthy that in Pasteur's experiments the attenuation of the anthrax bacillus could be pursued to such a degree that finally it lost virulence for all adult animals, even the mouse, and at the stage when it had lost virulence it had no immunogenic action because it had lost the property of establishing itself, in any degree, on the tissues. In other words, the organism had become apparently completely avirulent and non-immunogenic. Even at this stage, however, virulence could be recovered if the organism was inoculated into a newborn mouse (or rat). In the much more highly susceptible tissues of the animal which had not acquired properly the capacity of living an independent extrauterine life, the organism proved virulent. Progressive passage afterwards among increasingly less

susceptible species brought about "exaltation" of virulence, so that eventually the organism could be restored to its normal virulence as a bovine pathogen.

We may now review in the light of the above pioneer experiments some of the more recent developments on similar lines, taking again as our example, on account of its importance as the most severe known cattle disease and its importance to Egypt, that of cattle plague.

Since the closing year of the last century, active immunisation came to be practised, first of all in the scene of the devastating epidemic in South Africa, using, following indications derived there from Robert Koch's experiments in 1897. The fully active natural bovine virus ("virulent blood") was inoculated and the reaction which would ordinarily take place after inoculation with it was repressed by simultaneous inoculation of a suitable dose of antiserum (the Kolle-Turner, "serum-simultaneous" or "double" method). In some territories including Egypt during the next twenty years or longer this system was widely adopted, though in other territories, and particularly in India, it never gained widespread favour. This was partly because of the grave risks run from the employment of the fully virulent virus in the system of inoculation, partly because of the danger of accidentally inoculating parasites, notably piroplasms and trypanosomes, in the virulent blood, and partly because the reactions produced, which were often severe, very often brought about a lighting up of dormant internal parasites, such as piroplasms of the Babesia type, in the general circulation and coccidia within the bowels.

For these and other reasons, after the discovery of the Japanese workers (Kakizaki *et al.*) in 1916 eventually became known that immunity could actually be set up against the disease after the use of an inactivated tissue vaccine, their method tended to become increasingly popular, with the advocacy of several workers who believed they had effected notable improvements, in several territories. Field workers, and even the cattle owners themselves, did not invariably gain real confidence in this newer method, and its actual limitations have now been fully discussed in the preceding section.

Some attempt had actually been made by Koch himself while in South Africa in 1897 to modify the virulence of the virus by a few passages in sheep and goats, exaltation taking place after passage in the former species and attenuation after passage in the latter species. We now know, however, that hardly any change in the virulence of the virus could have been achieved in the course of a few passages.

Much later, in Indo-China, the French worker, Schein, studied the effects of passage in goats, at first being inclined to believe that inoculation with highly virulent blood from the goat would solve the question of excessive virulence as well as free the virus from contaminating protozoan parasites. However, in that territory inoculation with live virus derived from the goat never gained any appreciable use in current practice, and in the hands of Schein's successor, Jacotot, immunisation by means of formalin inactivated vaccine as orginally advocated by Curasson in French North Africa, was studied exhaustively and vigorously pressed forward in practice.

Real progress came to be made beginning in the year 1922 when Edwards in

India studied the effects of adaptation of the cattle plague virus to species other than cattle (including buffaloes). His first attempt was made by passage of the virus in rabbits. In this species, infection, when it became established in them, was marked by the occurrence of a very distinct thermal reaction, without any other symptoms. He had been inspired to seek for this kind of reaction through the researches of Weil and Breinl on the reactions produced by the inoculation of the causal agent of human typhus into guinea-pigs in which a "febrile, symptomless" reaction only was produced. Well-marked attenuation of the virus was observed to occur after passage in the rabbits, as evidenced by the far smaller dose of a potent anti-serum that was required for use in the double method of immunisation when rabbit virus and not the natural bovine virus was inoculated. However, upon the rather highly susceptible Himalayan hill cattle the rabbit virus never became completely safe for use by itself, even after nearly 15 months' intensive passages in local rabbits, after which the strain was lost through an outbreak of rabbit septicæmia among the rabbit stocks. Difficulty was experienced afterwards in fixing the virus on rabbits, and so attempts were made to fix the virus upon presumably more convenient animals in the shape of the small herbivora, sheep and goats.

Various workers had experienced varying degrees of success in establishing the bovine virus in the tissues of the goat, some having experienced no difficulty while others had failed entirely. The causes of success or failure are much clearer at the present day, for it is realised, as in the case of foot-and-mouth disease virus, that strains may arise within a given immunological type of a virus that develop a very high degree of affinity towards one particular host species while concurrently losing affinity towards other species. In the case of the strain of the cattle plague virus maintained at Muktesar (the Indian central veterinary research station) it had been maintained artificially by passage through cattle only in the laboratory for about 30 years and had presumably therefore lost almost entirely any affinity it had originally had for species other than cattle in becoming adapted for a long period to cattle only. Great difficulty was therefore experienced in establishing it in the tissues of either sheep or goats.

Something similar to the Pasteurian experience in restoring virulence by establishment in the tissues of the very young was forthcoming at the time from the researches of Beller and Tahssin Bey at the Reichsgesundheitsamt, Berlin, upon the causal organism of contagious bovine pleuro-pneumonia. As is well known, workers had found it impossible to reproduce, in the laboratory, symptoms and lesions resembling those of the natural disease in the bovine by artificial inoculation. After subcutaneous inoculation of the adult animal, there developed at the site of inoculation a more or less severe œdematous inflammation, which sometimes became so severe as to lead to death, but the characteristic leisons in the lung of the natural disease never arose. Beller and Tahssin found on inoculating several sheep with a cultivated strain of the causal organism that had lost even the property of causing marked local inflammation in these animals that a few of them retained complement-fixing antibodies in their blood serum for a long time after inoculation. These sheep which showed evidence of persisting infection were all pregnant ewes, which failed, however, to carry their fœtuses to

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full term. *Post-mortem* examination of the aborted fœtuses disclosed lesions in their lungs identical with those of the natural pleuro-pneumonia and from these lesions the causal organism could be isolated in pure culture.

After inoculation of the pregnant mother, therefore, the causal organism had traversed the uterine placenta and established itself in the far more susceptible tissues of the unborn lambs, there to set up pulmonary lesions which resulted in death and premature expulsion from the uterus.

In November, 1926, therefore, on return to Muktesar, Edwards operated on a pregnant goat so as to introduce the cattle plague virus (in bovine virulent blood) direct by inoculation into the fœtuses *in utero*. The virus in this way gained such a rapid foothold on their tissues that they were aborted within the next two days. Afterwards, the mother showed a slight fever, and blood taken from her and inoculated into other goats and cattle proved the presence of the virus in the blood. Passage successively afterwards through goats brought about such marked exaltation in virulence for goats that from the 12th passage onwards for a few months a very large proportion of the goats died, showing, strikingly, lesions of broncho-pneumonia, instead of the usual gastro-intestinal lesions observed in cattle. The virus, therefore, during early passage, had acquired pneumotropic affinities prominently, instead of the usual enterotropic affinities. Afterwards, in later passages, it lost these striking pneumotropic affinities and became prominently enterotropic and somewhat less virulent, so that a large proportion of the goats recovered. Towards the end of 1927, the virus, having thus been subjected to nearly a hundred passages, in the case of one strain, direct through goats only, and in the case of another strain, alternately through goats and cattle, was found to have undergone a striking *mutation*.

Whereas the original bovine strain killed almost all of the rather highly susceptible Himalayan hill cattle upon which it was tested, the goat-passaged strain (both direct and alternate) produced more or less well-marked symptoms of cattle plague, from which, however, they recovered. Afterwards they were found to be immune against the original highly virulent cattle strain.

During the cold-weather season of 1927-28—at which season "double" inoculations against cattle plague were usually carried out on the more valuable cattle kept at the Government Military Dairies and other institutions—it was decided to adopt the goat virus instead of the cattle virus at a few of these institutions under close supervision. At one of these—Karnal, in the Punjab—it was found that even when the goat virus was inoculated alone (into "controls"), that is, without a simultaneous dose of anti-serum, the cattle showed no more than a slight thermal reaction, and were afterwards immune.

It had long been known that the Plains cattle ("Zebu," *Bos indicus*) of India were naturally far more resistant to cattle plague than the Himalayan hill cattle, which were again a good deal more resistant than imported European breeds of cattle (*Bos taurus*—straight-backed cattle). From the doses of anti-serum required to confer passive protection, the ratios of relative susceptibility were found to be approximately 1 : 18 : 40, for the three classes of cattle. However, even among the Plains breeds considerable variation in susceptibility was known to occur, the

Ongole breed of Madras, for example, showing a relatively high degree of susceptibility.

Hence, where cattle were found having a genetically high threshold of resistance towards cattle plague, goat virus, derived from about a hundred passages in goats, could safely be used alone for the immunisation of those cattle: in other words, there was no need to inoculate a simultaneous dose of anti-serum as in the old "double" method.

Soon afterwards, field veterinary workers in India derived a considerable experience in the use of the newer goat-virus method from actual experience. The use of serum alone upon cattle exposed to infection in the scene of outbreaks was abandoned in favour of the goat-virus method, because it was found that protection against virulent infection arose very quickly after inoculation. During the next few years, this experience mounted to observations covering several million cattle, and cattle plague ceased to be the deadly menace it had always been to the livestock economy of India.

It was reserved to Pfaff, in Burma, however, to prove experimentally that following inoculation with the goat virus, cattle were usually completely resistant to inoculation with virulent bovine blood within 48 hours. The rapidity with which immunity was set up was in striking contrast with what was known to occur generally after inoculation with inactivated vaccines, where (as has been already said) after a negative phase a period of some days—9 to 12—elapses before the positive phase reaches its peak and the inoculated host is adequately protected. This rapidity of protection would represent an enormous economic advantage where cattle have to be quickly protected in the vicinity of actual outbreaks and the cost of inoculating them with anti-serum, to obtain passive protection, is prohibitive. This phenomenon of rapid protection against a "stronger" virus by inoculation a "weaker" virus has now become known as the interference phenomenon.

Later researches have shown that: (a) while in becoming adjusted to the tissues of the goat, goats infected with goat virus can readily transmit severe infection from goat to goat by contact, cattle inoculated with goat virus are unable to transmit infection either to healthy cattle or to goats kept in their vicinity; (b) the immunity following upon inoculation with the goat virus lasts several years, probably throughout the whole lifetime of the bovine animal, provided it has passed the stage of early calfhood at the time of inoculation; (c) for use in the field it is most convenient to use it in the form of spleen pulp from reacting goats, which is then stored in the form of the "freeze-dried" powder kept in vacuo, and in this form it can retain its potency for a long time (two years at least) if kept in the refrigerator; (d) it is impossible to obtain a strain of goat virus that can be safely used alone upon cattle possessing a very high threshold of natural susceptibility, such as European breeds generally. If attenuation is pressed forward by passage in goats or other suitable animals so that the virus actually becomes "safe" for European cattle, then at that stage the virus has also lost the property of establishing itself in the tissues of those cattle, and they remain afterwards fully susceptible

to natural attack; (e) as a first requisite in undertaking immunisation against cattle plague within a given territory it is therefore essential before undertaking field operations to ascertain experimentally the threshold of natural susceptibility in the breeds of cattle living in that territory and then, provided goat-virus immunisation can be used safely at all, to select a strain of goat virus for imunisation that has been attenuated far enough by passage in goats so that it will not cause an excessive number of deaths or alarming symptoms in the inoculated cattle while at the same time it will not have become attenuated so far that it will fail to establish itself in a considerable proportion of the cattle.

The above exposition summarising the present knowledge upon current immunisation against cattle plague, as it has now been ultimately introduced into Egypt, needs some amplification so far as concerns the peculiar conditions of Egypt itself. Following Edwards's arrival in Egypt in April, 1946, on information being communicated to him that cattle plague prevailed in the country and that immunisation with inactivated vaccine had failed to give satisfactory results, strains of goat virus were obtained from the laboratories in which it is now being propagated, namely, Muktesar (India), Kabete (Kenya) and Vom (Nigeria), the last-named being known to be a more highly attenuated form of the Kabete strain which in itself is suitable for inoculation into the Zebu breeds of cattle of East Africa but is too virulent for West African cattle generally. The Kabete strain being first to arrive, tests upon it at the Abbassiah laboratory indicated that, at any rate, the goat-virus method could be adopted for the cattle of Egypt. A preliminary trial upon 66 Government-owned cattle, at Sids, however, showed that the reactions produced were, in a considerable proportion of cases, somewhat too alarming. Hence, the Nigerian strain, which had then arrived and had been corroborated at the Abbassiah laboratory to be considerably milder than the Kabete strain, was tried out on 177 publicly owned cattle at Minchat Asem, the results showing that the reactions, on the whole, were not nearly so alarming, and few lost condition noticeably as the result of the immunisation.

Though the general result was that the Nigerian strain was much safer for use in the field than the Kabete strain, it was most noteworthy that, in the preliminary trials in both cases, marked heterozygosity was displayed as among ordinary Egyptian cattle when individual variation in susceptibility to the cattle plague virus is viewed as a genetic characteristic. Among the uniformly well-kept cattle at the above institutions, although no actual deaths were recorded, several cattle reacted with such very well-marked febrile and other clinical symptoms that their obvious state of ill-health would have produced alarm in the minds of individual owners. On the other hand, a considerable proportion, more especially in the case of the milder, Nigerian strain, gave so little indication of any reaction that doubt would arise in the mind of an immunologist as to whether or not the goat virus had really become established in them. The simultaneous inoculation of anti-serum did not usually prove that its employment was warranted to control the intensity of the reaction, for it either had no such effect (as at Sids) or it completely blocked out establishment of the goat virus (as was proved in later observations upon the Jersey cattle of the American Mission at Assiut).

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Hence, what was needed, in the face of the grave emergency presented by the actual presence of cattle plague in the country, was the employment of a strain of goat virus that would become established in as large a proportion as possible of the cattle located within the perimeters of the outbreaks, while among them it would not cause alarming symptoms in an alarmingly high proportion.

It is possible that the Muktesar strain, which was the last to be received, would have best complied with the above desiderata. However, in the face of the pressure of request to apply without exhaustive experimentation the knowledge already forthcoming to circumvent the spread of cattle plague, the Nigerian strain was issued, prepared, largely in improvised conditions at Abbassiah. from inoculated goats, and issued as freshly prepared, in liquid suspension, kept in ice, immediately to field workers. No time was available for freeze-drying, etc., and, in any case, the massive experience accumulated in India had been forthcoming without resort to freeze-drying procedures. In this way about a third of the cattle of the country (i.e., about 300,000) were inoculated from the end of June to just after the middle of August, and, with few untoward events in the field, the epidemic was completely arrested in Upper Egypt, and, after some delay, also in Lower Egypt.

Hitherto, we have stressed only the importance of genetic differences within a given species as affecting susceptibility in such a way as to produce considerable variations.

There are at least two other factors which profoundly affect susceptibility within a given individual, namely, age and the state of nutrition.

It is a very deeply rooted popular misconception that the very young and the very old, the ill-fed and badly nourished, the sickly and unhealthy, are those which are most highly susceptible to disease. Careful experiment proves, on the contrary, that, towards several diseases, these are the animals that are found to be most resistant towards the establishment of an infection. This statement applies fairly generally to those diseases which are caused by the filtrable viruses. It also applies to several bacterial diseases, such as blackquarter in cattle, braxy in sheep, and lamb dysentery. Exhaustive work on this point has been carried out particularly in respect to foot-and-mouth disease infection (Edwards). In the case of that disease, the animal is mostly highly susceptible when it is in prime condition and at the prime of life. Very young or very old animals are relatively resistant. Badly nourished animals, such as guinea-pigs which are not given an abundant supply of green food but are fed mainly on bran and cereals, are very highly resistant. Animals undergoing an intercurrent infection, such as rats affected with *Salmonella gaertner* or *ærtrycke* infection or guinea-pigs affected with pseudotuberculosis caused by Pasteurella infection, are also very highly resistant. Animals kept in unfavourable conditions as regards temperature and humidity are also somewhat resistant. What holds in the case of foot-and-mouth disease also holds almost entirely in respect to cattle plague (Edwards). For example, at Muktesar, in carrying out careful tests upon the potency of brews of anti-serum, it was necessary to select only cattle of pure Himalayan race (so as to avoid as far as possible genetic variations in susceptibility), between three and seven years of age, and in well-fed, good condition, and keep them, while under test, well housed

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and sheltered from violent extremes and changes of temperature. Researches in England recently on cattle plague had also shown that when housed there, for convenience, two in a loose box, and one animal worried the other so as to deprive it for some time of sufficient food, it was the worried animals that proved insusceptible afterwards to infection with a mild virus or small concentrations of a stronger virus, whereas the "bullies" succumbed readily. If, however, a very highly virulent virus was employed these differences were not so striking and tended to vanish.

The impact of these phenomena was brought out disconcertingly in the course of the early work on cattle plague immunisation in Egypt in 1946. Ordinarily, a highly susceptible race of cattle had been imported from Cyprus for purposes of experiment and as virulent blood donors. Until towards the end of June these animals furnished very valuable information on the properties of the several strains of goat virus, but afterwards, however, while issues of the selected mild strain were being dispatched to the field, they did not react, although from the nature of the materials with which they were inoculated, it had been anticipated that they would have reacted. During that phase, in the months of July and August, these animals were tied up on lines exposed to the heat of the sun, fed only on dry food, comprising rice straw and some cereals, and were undergoing intercurrent infection with foot-and-mouth disease. In the field, in most localities, on the other hand, where the goat virus was actually inoculated according to directions, all gradations in reactions were discovered to be occurring among the well-acclimatised indigenous cattle, as had been estimated from the preliminary trials, and this would account for the satisfactory disappearance of the epidemic. Belief, erroneously, upon the dependability of observations at that season upon the unacclimatised Cyprus bulls led to employment, in other hands, against the experimental evidence, of the too highly virulent Kabete strain temporarily in the field, with disastrous consequences, as well as to the ill-fated trial of an avianised virus, attenuated according to some experiments carried out by American workers during this last war by propagation of the virus in the developing chick embryo. At the present time, the current procedure is that ordained by the results of the early experiments in 1946, and it represents probably the most important immunological contribution placed within the reach of the Egyptian livestock economy.

One important complication liable to arise in the course of cattle plague immunisation is the "lighting-up" of dormant internal parasites. In the older, "double" method, grave risk was often run through the accidental contamination of the virulent blood with piroplasma and, sometimes, in some territories, trypanosomes. Even, as in the case of goat-virus immunisation, when no accidental contamination of the virus in that way is possible, the risks from babesiasis and coccidiosis are very great.

Probably, all cattle in Egypt become carriers of Theileria parasites and a very large proportion also of Babesia parasites. To what extent the cattle are carriers of bowel coccidia parasites is at present unknown.

Within a territory in which these infections are endemic, the cattle, on the

whole, develop a high degree of "premunity" against them. That is, the indigenous cattle contract infection at an early age, when they are relatively resistant, recover, and while they are actually carriers, they are immune against further infection. It is not a true immunity, such as occurs after complete recovery from an infection and the disappearance of the infective agent from the body. Imported cattle, however, arriving in the endemic area at a later age, contract infection with the above protozoan parasites severely and often die.

In the "premunised" animals, nevertheless, the state of equilibrium reached between the host cells and the latent parasites can be upset in certain circumstances. Noteworthy among these circumstances is a cattle plague infection, which, though its uncomplicated course might have terminated in recovery, produces very frequently a violent disturbance in the above equilibrium. Cattle may therefore be observed to show symptoms, a few days after the goat-virus inoculation, of severe red water ("Texas fever") or of a diarrhœa and dysentery which is mainly attributable to lighted-up coccidia. The Theileria parasites do not seem to be readily lighted up in a similar way. While undergoing immunisation, therefore, it is highly advisable to observe closely for the appearance of redwater symptoms and as soon as these symptoms are shown, inject immediately an adequate dose of trypanblau or acaprin to destroy the Babesia parasites in the blood.

Pregnant cows that are undergoing a severe reaction during immunisation are also very liable to abort, owing to the affinity of the virus for the cotyledonary tissues and the tissues of the fœtus. It is therefore well not to attempt immunisation of cows during pregnancy.

Not all viruses or other infectious agents can be attenuated by passage through a host other than that in which they occur as virulent pathogens. The discovery of a host in which such attenuation can be effected is largely a matter of trial and error or of pure chance.

Even in the case of the cattle plague virus, where well-marked attenuation in virulence for cattle takes place after prolonged passage in goats, the contrary may take place after passage in some other species. The French workers in Cochin-China, for example, discovered that even after two hundred passages in the pig there was no attenuation in its virulence for cattle, but, on the contrary, well-marked exaltation. The attenuation which takes place again in it for ordinary cattle after goat-passage does not apply to all other susceptible animals, such as, in part, buffaloes and some species of deer. Attenuation for the original host by passage in other species is, however, a phenomenon which the immunologist must always keep in mind as a possibility in securing modification of virulence. Following his experience with the infectious agent of swine erysipelas, it was natural that Pasteur should attempt to transform the virulence of the so-called "street-virus" of rabies as present in the brains of rabid dogs into the "fixed virus" which he obtained after passage of the brain material derived from them through the brains and spinal cords of rabbits.

A strikingly important recent example of successful attenuation of an

infectious agent by passage in a host other than the one in which it caused serious losses is afforded in the case of fowl pox. Here the current mode of immunisation now consists in the inoculation of susceptible fowls by scarification into the skin with pigeon pox virus, which is a variant of the fowl pox virus derived by adaptation to pigeons.

That several pathogens cannot be subjected to attempts at attenuation in this way so as to render them useful as immunogens can be ascribed to the following causes :—

(a) *The pathogen may be highly specific for the host in which it sets up disease.* Examples are, among the viruses, those of swine fever and sheep-pox; and, among the protozoa, the whole group of piroplasmata. Hitherto, no success has been obtained in transmitting these pathogens into any other species than the particular one in which they are known to set up disease.

(b) *The range of host-susceptibility may be a very limited one, so that one has the choice of only a few species in which to ascertain the effects of passage.* Examples are, among the viruses: (i) that of human poliomyelitis, which is transmissible only to nearly related primates, such as the chimpanzee; and (ii) that of South African horse sickness, which, until recently, had been found to be transmissible to only one other species of animal—namely, the dog. Among the bacteria, the example of the glanders bacillus can, to some extent, be cited, because apart from the equidæ the whole range of herbivora, including cattle, is completely resistant, though in the laboratory some zoologically remotely related species, such as the guinea-pig, can be infected artificially.

Recent work has indicated, however, that the possible range of species-susceptibility in the case of a given pathogen may be far wider than was suspected when the development of clinical symptoms and pathological lesions was taken as the only criterion of transmissibility. For example, in the case of the human yellow fever virus, the application of newer techniques for detection has disclosed the possibility of this virus being present in several species of animals, including cattle and deer, throughout extensive areas in several parts of the world. Blood serum from a large proportion of the animals in these areas has revealed neutralising antibodies for mouse neurotropic strains of this virus. Psittacosis, which a few years ago was believed to infect only birds of the parrot family (budgerigars, etc.), has now been detected, using similar methods, among a wide variety of birds in localities believed to be entirely free from it; even the pigeons in Trafalgar Square, London, have been found to harbour the virus. A most significant example is that of bovine malignant catarrh: the symptoms displayed in this disease are those of highly febrile, alarmingly fatal infectious disease. Yet, transmission, even by close contact, to healthy cattle in the vicinity of the diseased animal does not occur. Recent work tends to show that sheep in the vicinity contract infection with the virus in an "occult" or "inapparent" form. Cattle afterwards are liable to contract disease only from such sheep. These examples suggest that in several diseases the transmission of infection to species other than those in which acute overt disease is set up may result only in those species acting

as "carriers" of the infection, which, when it is transmitted back again to the original host, sets up in it a very severe form of disease. The swineherd disease, studied recently in the South of France, is a particularly interesting example, where pig attendants have been found to contract severe infection from the pigs in their charge which, themselves, show no visible disturbances in their health.

Several other methods are, however, now available for causing variation to occur in a given pathogen. One of them depends upon the possibility of effecting change in its tissue-affinities. The viruses of human and swine influenza are said to be *pneumotropic* because in infected subjects their presence is restricted to the epithelium of the respiratory tract. The viruses of rabies, human poliomyelitis, encephalitis lethargica, and louping-ill in sheep are said to be *neurotropic* because in subjects showing the characteristic symptoms of these diseases they are present in very high concentration in the cells of the central nervous system, while other tissues may disclose either no virus or only small amounts—as in the saliva in the case of rabies—during the terminal stages of attack. Other pathogens are *dermotropic*, as in the case of the viruses of foot-and-mouth disease, contagious pustular stomatis in horses, herpes zoster, and the whole group of the variola-vaccinia viruses. Other pathogens may be predominantly *enterotropic*, while some are classed as *pantropic* because they are found throughout the body, with apparently no special affinity towards any tissue system. On close investigation, some pathogens may be discovered to have a very highly selective distribution among a particular cell system. For example, in the case of foot-and-mouth disease the virus can be found during the initial, febrile stage of attack throughout the whole blood. In the case of cattle plague, on the other hand, the virus is restricted to the leucocytes—and among these apparently to the lymphocytes only—while the blood plasma itself is highly toxic towards any liberated virus.

These selective tropisms are not restricted to the viruses only, but apply to the whole range of pathogens. A conspicuous example of an enterotropic bacterium, for instance, is that of Johne's disease in cattle (*Mycobacterium paratuberculosis*), and of a pneumotropic bacterium, *Brucella bronchiseptica*, in dogs.

The most striking immunological advance in the direction of attenuating a pathogen by modifying its tropisms was made by Max Theiler about 1930 at the Rockefeller Institute, New York, in the case of the human yellow fever virus, which in the body of the human subject affected with the acute natural disease, or of an experimentally infected primate, is *pantropic*. It is not possible, by the customary means of artificial infection employed in the laboratory—namely, subcutaneous, intramuscular, intraperitoneal, or intravenous inoculation—to transmit infection to animals outside the range of those primates most nearly related to man (as has been already said). Conceiving the idea that the natural defences of the cells of the central nervous system would be much weaker, Theiler tried the subdural route of inoculation, employing mice as his experimental subjects. At first transfer, a very small proportion of his mice developed paralytic symptoms. With succeeding transfers of brain and spinal cord material from affected mice, the proportion of mice which succumbed to these symptoms became increasingly higher, until in time the virus became well fixed as a mouse neurotropic strain. At

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this stage, it was discovered to have lost its pantropism for the higher primates, which, however, after subcutaneous or intradermal inoculation with it and passing through a very mild reaction, were found to be solidly immune against the original, highly virulent, pantropic strain. In the actual immunisation of human beings, during the subsequent early history of yellow fever immunisation, it was found to be a safe plan to inoculate people entering endemic areas with the mouse neurotropic strain suitably admixed with anti-serum, choosing the intradermal route for inoculation.

Advantage was taken of this discovery soon afterwards by workers (Alexander and others) at the Onderstepoort Veterinary Research Laboratory, South Africa, in solving one of the most baffling problems in immunological practice—namely, in producing a satisfactory immunity against South African horse sickness. Since 1900, when McFadyean discovered the cause of this deadly disease to be a filtrable virus (the second disease proved among man and animals to be caused by such an agent, the first being foot-and-mouth disease, found by Loeffler and Frosch to be caused by a filtrable agent two years earlier, in 1898), vigorous attempts had been made to protect horses destined for employment in endemic areas by resorting to methods comparable to those deemed most satisfactory at that time for conferring protection against cattle plague, of which it was generally thought that the serum-virus (Serum simultaneous, or "Double") method was the best.

Very great difficulties were, however, encountered. On the whole, it was found very much more difficult to immunise horses than mules or donkeys (which have a high degree of natural resistance) by means of a parallel method, the "hyperimmune" serum failing to protect against fatal attack a very large proportion of the horses simultaneously inoculated with the virus. Secondly, it was found that even when they appeared to have been satisfactorily immunised at the laboratory, they were by no means invariably protected, or "salted," against attack when they entered certain endemic areas.

To some extent, the grave risks entailed in the immunisation of horses against horse sickness were curtailed by using virus which had been passaged through dogs, which was a method claimed by Bevan in Southern Rhodesia to have given relatively safe and satisfactory results. The problem presented by the failure of horses that appeared to have been satisfactorily immunised in the laboratory to withstand natural infection later on when they were introduced into certain endemic areas was discovered to be due to the existence of more than one immunological "type" of virus. Later work revealed the existence of about six such "types" of virus in South Africa. This characteristic of "breaking up" into "types" is now known to occur with several viruses infecting man, animals and plants, of which well-known examples are the human influenza virus, the foot-and-mouth disease and equine encephalomyelitis viruses, and the potato-wilt virus. While the clinical symptoms and pathological lesions set up by all "types" of the virus are usually entirely similar, the types differ profoundly in so far as recovery from infection set up by any one "type" does not confer protection against infection with another "type." To obtain passive immunity against natural infection, it is therefore necessary to inoculate an animal with an anti-serum which is specific

against every known type, i.e. to use a "polyvalent" serum. In the "double" method, as practised in the case of horse sickness, the horse should be inoculated with all known immunological "types," or, at any rate, with all the "types" identified in the endemic area it was about to enter, as well as a "hyperimmune" polyvalent serum to prevent it succumbing to infection with each type inoculated. It can readily be appreciated that in practice this would become a perplexing and risky process.

Applying the principles discovered by Max Theiler in the case of the human yellow fever virus, Alexander and his colleagues in South Africa discovered that for each immunological type of the horse sickness virus they were able to produce neurotropic variants, first in guinea-pigs and later, and more satisfactorily, in mice. The immunogen thus consisted of mixed suspensions of the brains of mice that were succumbing to the several "types" of virus after intracranial inoculation. Inoculated horses withstood infection with this material without showing any serious symptoms and were afterwards immune against lethal infection with the natural pantropic strains.

This has been one of the most striking advances in immunological practice and it has now come to be widely applied in Egypt following the recent introduction of horse sickness into the country.

The virus of horse sickness is one of the most resistant kinds known, being able to survive, for example, at ordinary atmospheric temperatures in virulent horse blood, with a concentration of phenol added to it to check bacterial concentrations for an indefinitely long time. Great care to ensure viability, as in the case of the cattle plague and dog distemper viruses, which are highly "fragile," is therefore not necessary, and the mouse brain suspensions are now usually despatched as such to the field, in buffered solutions to which ether (5 per cent.) has been added and well shaken up with them to prevent bacterial contamination. The use of ether in this manner has now become well established in virological technique, and it can often be employed to "cleanse" a virus of accidental bacterial contamination, as when the infected crusts of suspected ruptured pox vesicles are received at the laboratory for examination.

(*To be continued*)

CAN SPEED AND STAMINA BE COMBINED?

By BARONESS WENTWORTH
Crabbet Park, Poundhill, Sussex

Of late there has been a melancholy wail of distress among English breeders at the apparent loss of stamina in our thoroughbreds and at the wholesale onslaught by French-bred and other foreign horses on our richest races, which we had come to regard as our national unassailable perquisites. This has been followed by a wild storm of queries as to the causes of their success. Is it their woolly nosebands or their trainers, their feeding or their blood or their jockeys? Where do they get those Arabian heads? Is it some cross of Arabian

blood or some secret drug that sends them streaking to victory from behind our exhausted horses with this exasperating ease, their triumphant woolly nosebands visible a mile off!

Personally, I cannot see what cause we have for complaint. We had marvellous stayers 150 years ago. Horses which ran three four-mile heats in one afternoon several times a week or month and were ridden inconceivable distances to race meetings as well and never turned a hair. Bald Charlotte carried up to 18½ stone, and as for that little rocket bomb, Atalanta, she ran heat after heat and her record is staggering. Some mares even made light of having a foal in their spare time. But since then our aim has been speed, not stamina, and gradually these 12-mile races grew shorter and died out. Size and speed increased and all our energies were eventually thrown into producing the greatest speed at the earliest possible age. The racing of yearlings lasted quite a long time before it was given up. Two-year-old racing continues in full force. We have bred for speed now for 150 years, and we certainly have got it. Our two-year-old speed is simply miraculous and not to be matched anywhere in the world. Records are constantly broken, but instead of being satisfied, we are surprised and vexed we cannot keep it up for miles instead of furlongs. Even to-day, Brown Jacks and other stayers are not to be despised, but as we systematically geld all our Grand National winners, we have only ourselves to thank if their priceless qualities are lost. Then there is the question of conformation. Do our Olympic Games match a human athlete sprinter against a marathon runner? Can we class a wrestler with a ballet dancer? Do these things require a different build and physique? It seems doubtful if horse breeders can combine them, and I am not sure that the attributes for super speed are not only incompatible with, but antagonistic to, super staying power. The impetuosity of lightning movement is not the same as the persistent endurance of long continued effort, and certainly it cannot be done on our present system of breeding or keeping. We sap all the vitality from our two-year-olds, often break their hearts, strain their muscles, jar their feet and overwork them to staleness. Staleness is a deadly enemy to staying power.

Again, we are inundated with endless mathematical paper calculations based on what is called staying blood and sprinting blood, but we find Mahmoud winning the Derby in spite of them, and sudden outbursts of speed appear where least expected. What is the secret of the foreign supremacy? Can a new Arab cross have crept in somewhere? Frankly, we do not know. Stud books show that Italian, French and American blood are all founded on our own blood, with an occasional unknown flaw of slow cold blood in all the pedigrees, including our own. The difference is infinitismal and negligible in the 67,108,864 units of some of these pedigrees. Bruce Lowe did some interesting work, but conclusions based on his numbers are quite unsound. For one thing, his numbers for the most part merge into each other when taken back further, so that families which are announced by some writers as moribund, extinct or inferior are very much alive, e.g. No. 36 (which is the same as 9) and 31, 35 and 40, all of which go back to 4. I have reduced the 43 families to 18 all told. The influence of one mare calculated in direct female line is more than doubtful. They are not even

as useful as the female Arab strains, which, at any rate, indicate famous owners or districts of pure origin.

The influence, however, becomes a matter of great importance when the bulk of the pedigree is saturated with re-crosses of one particular mare, as in the case of the Arabian mare, Old Bald Peg, No. 6, registered long before Weatherby's G.S.B. existed, as Arabian mare by an Arabian horse from an Arabian mare and whose blood is repeated in our pedigrees to the tune of hundreds of thousands of re-crosses, which are doubled and reinforced in each succeeding generation instead of weakening. We, further, have in Sir Ralph Milbanke's collection a picture of the mare and of her sire, whose name we find was Sultan.

The latest refuge of prejudice is to discredit the early artists as unreliable because they drew impossibly small Arab heads. Surely the feeblest of quibbles! No man could invent what he never saw and however stiff the drawings, the small head and high-carried tail confirm the Oriental pedigrees.

In the case of the Tregonwell Natural (so-called) "Barb" mare, No. 1, we find she was originally registered as an Arabian, that her name was Snorting Bess and her picture from the Milbanke collection shows a typical grey Arabian of high class. Owing to the inevitable intensive inbreeding of all the early sires and mares, it cannot really be contended that any of our racing strains are, in matter of solid fact, more full of staying blood than others. All Oriental blood is staying blood, and, in fact, *on paper* there is nothing else, and we see reversions to the Barb convex head as well as to the Arabian head. The common coarse types can only be accounted for by the unrecorded blanks in our foundation line, which are the only possible source of origin. Staying power would therefore seem to depend not so much on blood lines as on conformation, and the French return to power might well be a reversion to type in more than head. The worst of it is, that with few millionaire exceptions, breeders are unable to choose the sires which suit their mares. It is useless to write learnedly on how to mate and combine pedigrees when breeders cannot get nominations and have to take what they can get, knowing (as I myself often do) that the conformation of the sire is utterly unsuitable to the mare I am sending to him. Even a Derby winner is not suitable to every mare.

The syndicate principle of fantastic valuations, forced on us by taxation of stallion fees, is the worst possible one for the breed. It enables a man with money to pay £4,000 for a share (this is the latest) and send any mare, however unsuitable, while many good mares remain unable to visit anything but third-rate sires because their owners cannot afford outrageous fees. What is the use of a National stud if its sires are out of reach of the public? The ballot is, I understand, merely from a limited number of already selected mares. It recalls our National ownership of the mines and inability to buy coal.

Then there is the other question, i.e. the over-racing of two-year-olds and the lack of food for brood mares and yearlings. There are exceptions, of course, but the condition in which brood mares now usually come back from stud visits, even to famous studs, is deplorable, with ribs bare and coats staring mere skeletons of their former selves. Sometimes they are even infested with vermin and scarred with marks of kicks, and very often are not in foal. Lack of

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pasture, shortage of rations and labour all contribute to this serious state of things. Far too many visiting mares are crowded into one paddock, which gets horse sick. The stud grooms cannot get good men and trying the mares is left to chance. How can we hope to breed good foals with their dams in this condition? The foals are born lacking in vitality, from the start, the quality of the mares' milk is poor, and, as yearlings, too, they do not get enough food.

If we are not to fall back from the front rank of breeders, we shall have to remedy this state of things without delay, or America will sweep away the races which France has not already secured and the duel for supremacy will be between these two countries.

The main allocation of rations should be to breeders, even at the cost of cutting them for second-class racing geldings and platers, which are of no value to breeding whatever.

The next question of the hour is how to introduce more staying blood. Could Arab blood be reintroduced? Those in favour of giving the possibility a trial are usually reasonable, but are immediately violently attacked by people totally reckless as to facts and having not the smallest idea of the enormous gap between the classic Arabian and the hack Arab of the East, and who are in no position to express any opinion whatever. They know nothing of their subject and are apt to become gratuitously insulting to those who do, and display their ignorance by boasting of a trumpery private match with no stakes run fifty years ago in which a second-rate thoroughbred defeated a fourth-rate so-called Arab, which is periodically produced as front-page news and is given the importance of an international Test Match regardless of the (invariably suppressed) fact that the so-called Arab was afterwards defeated as easily by Arabs as by the thoroughbred. I cannot imagine why they are so proud of it, as the horse's merits or demerits have no more to do with Anglo-Arabs or the horses of to-day than the death of Queen Anne. It would be more to the point to quote the scores of races *won* by Anglo-Arabs against thoroughbreds in England, Australia and on the Continent, and we never hear a word of the 17 thoroughbreds in an official 310-mile race that failed to get half way over a less severe course than that easily won twice by Arabs.

Nobody in his senses would contend that a pure Arab could live with the thoroughbred sprinting speed of to-day, but neither could any sane person deny that the Arabian always has been, and still is, the fastest of all unspecialised breeds and is still the world's greatest long-distance weight carrier of all breeds. There is no evidence to suggest that he has lost his potentialities as a sire of racehorses. If the Darley Arabian could race a Derby winner to-day, he would be outclassed, but this is not to say that the Derby winner does not owe his existence to the Darley and hosts of other Arabians.

Perhaps, therefore, there is as much sense as nonsense in the query: "Could Arab blood be reintroduced"? The question as to whether it *can* is another matter and whether it *will* is an almost certain negative. Old Mr. James Weatherby definitely thought it could, but only after pure Arabs of chosen blood had been improved in England over sufficiently long a period to produce size and scope. Hence the Arabian section of the G.S.B.

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Anglo-Arabs have been bred for many years past very successfully in Australia and France and have won hundreds of steeplechases and a good many flat races. In France especially, there has been great enthusiasm for Arab blood in varying mixtures ever since the days of Napoleon, the chief obstacle being the perpetual breaking up of studs by wars.

In Australia some big races have been won by the third generation in descent, and I am bringing out a booklet with all the statistics shortly. Until the era of Ben d'Or, the times of Arab and English racing were not such poles apart as they now are, but since then speed has made a tremendous advance, leaving the Arab completely outclassed. How could any unspecialised breed compete with the product of intensive specialisation over 150 years? If it could do so, there would be no sense in specialising for anything. The breeds stand at the opposite ends of the racing scale. Arabians are as supreme over all breeds in staying power, soundness and weight carrying as thoroughbreds are in their miraculous speed. Each in its respective department can give away almost unlimited weight and leave the other stranded the wrong end of nowhere. In theory, it would seem easy to combine the two—in practice, time and money, and an extreme scarcity of improved Arab material are prohibitive. The specialised thoroughbred has been improving for centuries. The desert Arab has either remained in *statu quo* or deteriorated, for in Arabia all conditions are more and more adverse to horse breeding and the numbers and quality of desert-bred horses are no longer what they were fifty years ago.

Only in England are there improved Arabs bred to a size and conformation suitable for any experiments at all if the produce is to rise beyond the hurdling chasing and hunter class, where its merits are already known. An initial loss of speed is inevitable and nothing short of an Oaks or Derby winner or a first-class sire of sprinters mated to selected individuals from proved world's record endurance strains could possibly be of any use. Experiments would have to be on a large scale, for even classic winners mated to other classic winners do no always get winners, and there are thousands of classically bred T.B. mares which never get even the winner of a selling race. It is quite certain that no one is going to mate an Oaks winner to an Arab at the present fantastic selling values of her produce, and the number of Arab mares fit to send to a Derby winner could be counted on the fingers of one hand, even if the owner of a Derby winner could spare a nomination, for which hundreds of T.B. breeders stand waiting in queues, so the whole idea can be ruled out.

That it is *possible*—yes. That it is practicable—no.

What, then, are we to do about it? My only suggestions are: (1) Feed our brood mares and young stock at all costs. (2) Go easy with our two-year-olds. (3) Give less importance to "paper" breeding and more attention to conformation. (4) Stop the syndicate sequestration system. (5) Adopt the American stallion owners' "living foal" clause, at any rate for proved brood mares, instead of insisting on "the pound of flesh" system exacted here, regardless of whether failure to breed is the fault of the stallion management or not. It would stimulate indifferent owners or careless stud grooms to take more trouble

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with mares in their charge and encourage small owners who cannot afford the loss of a 300 to 400-guinea fee for nothing.

The record of proved brood mares and the testing of others should eliminate any danger of the mare being at fault.

STUDY ON MICROCOCCI

By H. FARRAG, B.V.Sc., M.V.Sc.(Cairo), Ph.D.(Kansas)
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OWING to the fact that micrococci were found in very high percentage in both normal and mastitis milk in this piece of work, it was believed that the study of these micrococci as regards their incidence, identification and the in-vitro action of penicillin might be of value.

Evans (1916) made an extensive survey on the bacterial flora of cow's milk and found that the percentage of micrococci was 39.2, 46.9, 47.5, 72.5 and 75.7 in different dairies. Altogether, micrococci were isolated from 58.9 per cent. of samples examined. Copeland and Olson (1926) made a similar survey and found that most of bacteria in normal milk were micrococci. Borner (1930) found the percentage of these organisms between 82.5 and 100 per cent. of the examined samples. Plastridge (1939) obtained similar results and found that micrococcus lactis varians was the most frequent in freshly drawn milk samples.

An attempt was made to determine:

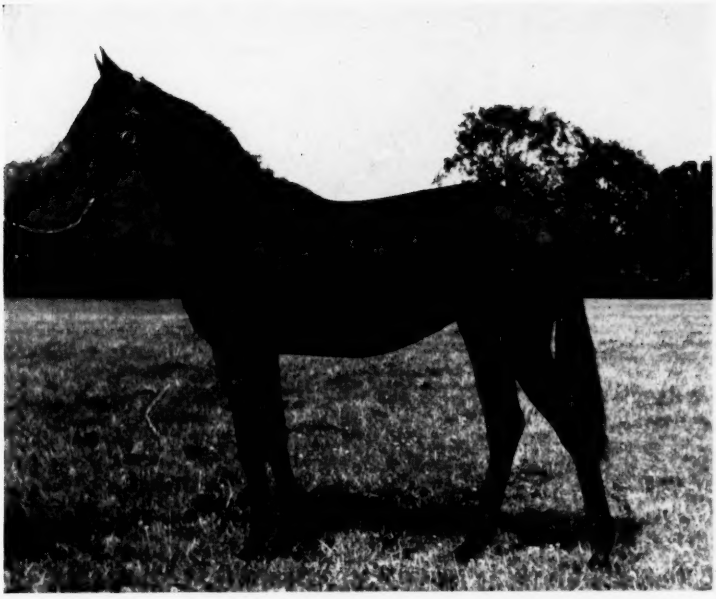
1. The different species of micrococci found in the udder.
2. The percentage of these organisms in milk samples.
3. The in-vitro action of penicillin on such organisms.

The following procedure was used to determine the different species of micrococci found in the udder:

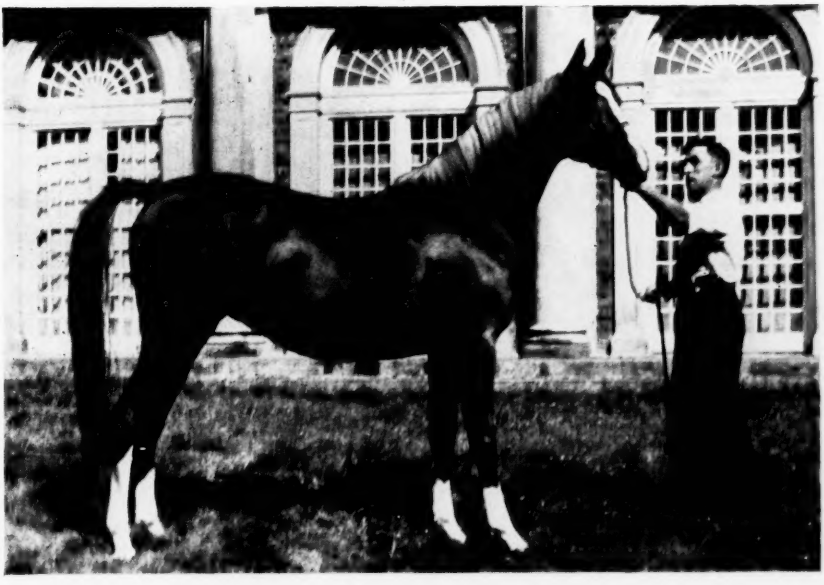
Samples of milk were taken asceptically from cows. These were thoroughly shaken, 1.1 ml. of milk removed, of this 0.1 ml. was drawn into a sterile petri dish, the remainder 1.0 ml. was diluted with 99 ml. sterile water and 1.0 ml. of this dilution placed in a petri dish so that the final dilutions would be 1 : 10 and 1 : 100 respectively. Into each of the petri dishes was poured about 10 ml. of agar, to which 5 per cent. of sheep blood was added at 45 deg. C. The plates were incubated 24-48 hours at 37 deg. C. Smears from 100 colonies were examined microscopically; 95 of the colonies examined were of gram positive cocci; five only were found to be gram negative cocci. Growth of colonies on the plates was quite luxuriant, all were non-hæmolytic. The percentage of micrococci in milk samples was found to be 90 per cent.

They were identified according to Bergey classification from the biochemical reactions obtained, as follows:

Micrococcus	epidemicus	20%
„	varians	13%
„	caseolyticus	11%
„	conglomeratus	8%
„	luteus	9%
„	candidus	7%



Anglo-Arab yearling filly, Arab Sue. Thoroughbred mare.



Arabian three-year-old filly. Seven generations bred in England.

(Article by Baroness Wentworth.)

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The metallic object: A tin lid ⅛ in. thick.

(Article by W. S. Scott.)

Micrococcus freudenreichii	7%
„ aurantiacus	7%
„ flavus	5%
„ candicans	5%
„ mucofaciens	3%
Unidentified gram negative cocci	5%

The in-vitro action of penicillin on micrococci: The sensitivity of these organisms to penicillin was determined by the Standard cup-plate method, i.e. agar plates were seeded with these organisms, allowing a few cubic centimeters of seeded agar to flow over the surface of the agar plate and draining off the excess. The glass cylinders used in the assay of penicillin were placed on the agar, filled with penicillin of different dilutions in each plate (0.1, 0.5, 1 and 5 units penicillin per ml.). At the end of the incubation, zones of inhibition were measured and thus the sensitivity of each organism was determined. From the data collected it was found that all the isolated micrococci were sensitive to penicillin, although the sensitivity varied somewhat in the different strains.

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CLINICAL ARTICLE

OSSEOUS ACTINOMYCOSIS BOVIS: A Mistake in Diagnosis

By WILLIAM SCOTT, D.M.V., F.R.C.V.S., F.R.S.E.
Bridgwater.

Some months ago I was called to examine a Devon heifer which was not doing well. On examination, nothing specific was noticed except that the beast was "light" in condition. I drew the owner's attention to a hard swelling on the right upper jaw, above the cheek. He said he had noticed it previously. The lump was hard and fixed to the bone. I suggested an operation after the beast's general condition had been improved by a course of tonics and extra food.

Subsequently the animal was cast and anæsthetised using chloroform. An eliptical piece of skin, approximately two and a half inches in length, was removed and the underlying connective tissue dissected free in order to use a trephine. On using the latter instrument I felt a distinct grating which was more metallic than osseous. On further dissection a piece of tin was detected. I tried to remove this with artery forceps but failed and had to use pliers and considerable force. When the foreign body was removed frothy food and air rushed out of the wound. The area was cleaned up and the wound was satured firmly using three deep and three superficial sutures.

Four days later the the sutures gave way and food again issued from the wound. This time I inserted four button sutures and gave instructions that the

diet should consist only of sloppy food i.e., bran cake and crushed oats, and straw bedding eliminated. In seven days the wound was healed .

REMARKS.—I have operated on several thousand cases of Actinomycosis or actino bacillosis and I have never made such a diagnostic *faux pas* before. Other points noticed were there was no salivation and the mouth and oral breath were quite sweet. When the tin was removed there was no sign of sepsis. Was this due to the peptonising effect of the ptyalin of the saliva on the metallic body which in turn may have liberated a bacteriocidal element?

The foreign body proved to be the end of a round tin such as is used in the food canning industry. In the corner of the field in which the animal grazed there was a district council refuse dump.

REVIEWS

The Bloodstock Breeders' Review, Vol. xxxv, 1946. The British Bloodstock Agency, Ltd., 26, Charing Cross Road, London, W.C.2.

PREVIOUS issues of this well-known annual have gained such a high place in public esteem that when we say that this volume is up to standard, no better tribute could be given.

In the story of the racing year 1946, we see the first signs of the "French" invasion, which since then has gone on to full flood. Racing in retrospect is always intriguing, if only for the reason that one can get a new line on the failure of the horses which were fancied at the time. As usual, there are accounts of racing and the stud in many countries other than England.

The statistical tables provide a great mass of valuable information and cause one to marvel at the diligence and persistence of the compilers.

It is noted that more stallions are being syndicated. Is this system likely to be to the ultimate good of the industry? There is much to be said for and against. May we suggest that an informative article on the subject would be a welcome addition to a future issue.

On the whole, 1946 was a boom year for the thoroughbred, his owner and trainer. Will this state of affairs last? It is questionable if continued boom conditions are good for any man or business. Easy money means high prices and high prices, in the end, mean retraction. The T.B. industry will be sounder and healthier when things become more normal from the financial point of view.

One cannot leave this review without reference to E. E. Coussell, notice of whose death on December 28, 1947, is conveyed in a slip inserted in the book. Mr. Coussell was largely responsible for the initiation and subsequent publication of the "B.B.R." For many years he bore the main burden and, indeed, at the time of his death, he was in the midst of his work on this volume. His unique knowledge of pedigree and of all that appertains to the T.B. and racing, and men and affairs, coupled with an extremely gifted pen and an alert and retentive brain made him the ideal editor. He was a veritable encyclopædia to whom one could turn in any dilemma. He was courteous, sage and kind.

We have said on previous occasions that the "B.B.R." is the *vade mecum* of the thoroughbred world, and this volume confirms that opinion.

Index of Treatment in Small Animal Practice. By HAMILTON KIRK. London: Baillière, Tindall & Cox. Pp. viii + 769. 149 illustrations. Price, 40s. net.

"IN no other than this textbook can detailed information be collectively found of all the basic methods. . . ." This is the claim of our author in regard to the methods of treatment, and it is justified.

The book is divided into three sections. Part I deals with the methods of treatment now available. Those wave-lengths of the spectrum available to the practitioner in light, heat and electric currents in general are covered in 20 pages and form a clear introduction to the subject. Those specifics that have of recent years undergone the polysyllabic dub of chemotherapeutic agents have approximately the same amount of space. Hormone therapy takes 50 pages. Vaccines and sera take up only 10 pages, as do vitamins. Sedatives, anæsthetics and antiseptics occupy 30 pages.

We find in this relative allotment of space by an author considerable interest. In part it shows us the relative importance placed by an experienced practitioner on the varying parts of his armoury, and in part it shows the influence of contemporary fashion and thought in modes of treatment. The predominance in pages given to hormones as against vaccines is intriguing and unexpected in the eyes of your reviewer, though the idiosyncrasy may belong to the latter and not the author.

Part II is an index of treatment. We have subjected most of the items to critical survey, and on the whole this section emerges with great credit and may be safely followed by anyone seeking therapeutic guidance. Points to which the author might apply himself when the next edition is called for are, in particular, the sections on lice and fleas. There is far too much space given to obsolete methods. However interesting these may be historically, they only act as a screen to the reader who wants to select the best and most up-to-date method. So we would counsel a drastic cut here. After all, gammexane is a complete answer to all skin parasites, with D.D.T. a good second, and all the rest lengths behind. The same applies to the section on mange.

In the matter of bee stings, let Mr. Kirk study some of the modern textbooks on bee-keeping and learn the value of adrenalin in severe cases of shock due to multiple stings.

Masturbation in the dog can often be cured for a long period by emptying the prostate. It appears that tension in the latter organ is the begetter of the vice in the male. The pet dog that rides the human leg often stops this embarrassing habit entirely after one treatment. But, on the whole, there is little to find fault with in this section.

Part III deals with special techniques, such as handling, feeding, nursing, giving medicines, destruction of rats, and euthanasia of pets. On page 639, the hoary old picture of Hobday's operating table persists. This mass of keyholes is a thoroughly reprehensible piece of apparatus, in our view. The slots become the repository of dried discharges and dirt. Even a kitchen table is preferable to this article, and we recommend its deletion in future editions.

The chapter on euthanasia might be said to be slightly out of date in regard

to electric euthanasia. This is a pity, for this book could do much in educating the practitioner and encouraging him to adopt this clean and rapid method. There is still far too much of the " prussic acid is good enough for me " attitude towards euthanasia amongst a proportion of practitioners.

We should welcome a reasoned essay on this topic of small animal euthanasia with guidance as to the method of choice with each species of small animal.

To sum up, the book is an excellent " under the counter " companion, that will improve with age as do all good vintages. We shall study the second edition with interest, and we hope soon.

NOTICES

C. J. HEWLETT & SON, LTD.

We are informed by Messrs. C. J. Hewlett & Son, Ltd., that they are now able to supply Calcium Boro-Gluconate Injection, with the addition of phosphorus and magnesium, under the name of Calcium Boro-Gluconate P-M. They state that this amount of phosphorus present in the solution is half the calcium content and that this proportion is identical to that normally found in the blood of the cow, so that injection may be made either subcutaneously or intravenously.

As the inclusion of magnesium in solutions of this type is useful for its effect in stabilising the calcium in the blood stream, Messrs. Hewlett & Son claim that the improved formula, which may be used for any calcium deficiency, is indicated particularly to prevent relapses in milk fever cases.

They also have a second formula, available under the name of Calcium Boro-Gluconate P-M-D, which has the addition of 20 per cent. of dextrose. This, they suggest, would appear to be indicated in cases of milk fever complicated with acetonæmia.

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